

# INTRASPECIFIC VARIATION IN HERBIVORE DEFENSE: THE EFFECTS OF DEPTH AND ULTRAVIOLET RADIATION ON SUSCEPTIBILITY TO HERBIVORY OF TWO ALGAL SPECIES

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**Abstract:** Defense against herbivory is important for algal survival in a coral reef environment. Different types of morphological and chemical defenses may be employed by different algal species depending on factors such as resource availability or herbivore pressure. We examined intraspecific variation in herbivore defense along a depth gradient in the defenses of two brown algae species, *Lobophora variegata* and *Dictyota divaricata* by assessing their susceptibility to herbivory. *L. variegata* individuals from 15 m experienced less herbivory than individuals from shallower depths in trials at 5 m but the trend was not statistically significant. Individuals from deeper water may be better defended because the costs of herbivory are greater in resource-limited environments despite lower herbivore pressure in that environment. Also, *L. variegata* may be relying more on morphological defenses than chemical defenses to deter herbivory. The opposite pattern of herbivory was observed in *D. divaricata*: individuals from deeper depths were significantly more susceptible to herbivory than individuals from shallower depths. This may indicate that this species only employs defenses when herbivore pressure is great (i.e., in shallow water), or when carbon does not limit growth and reproduction. A study of the effects of ultra-violet (UV) radiation on *D. divaricata* showed greater herbivory on plants after exposure to UV radiation. This suggests that UV exposure may reduce the defensive capabilities (e.g., lower concentrations of secondary metabolites) of *D. divaricata*. This trend was more pronounced in algae from 15 m than from 5 m, which may be due to lower levels of mycosporine-like amino acids (MAAs) – compounds that have been shown to protect algae from harmful ultraviolet radiation – in plants from deeper water. Overall, it appears that intraspecific variation in herbivore defense is species specific and that general patterns are not present in marine algal communities.

**Key Words:** algae, *Dictyota divaricata*, *Lobophora variegata*, MAAs, secondary metabolites

## INTRODUCTION

Herbivory has a profound effect on the distribution and abundance of macroalgae in marine environments (Hay 1981, Hay and Fenical 1988). This is particularly true on coral reefs where 60 – 90 % of total algal biomass may be removed by herbivores (Morrison 1988). However, herbivory rates on macroalgae can be reduced by the production and accumulation of secondary metabolites (Hay and Steinberg 1992). Concentrations of secondary metabolites may vary between species (Hay and Fenical 1988) and within species (Hay 1996). Understanding within-species variation in the production of secondary metabolites and its effects on plant-herbivore interactions has important implications for ecological and evolutionary patterns and processes in coral reef ecosystems.

Multiple frameworks have been proposed to explain intraspecific variation in the production of secondary metabolites (Hay 1996). The spatial-variation-in-herbivory model (Hay and Steinberg 1992) assumes that the production of chemical defenses is costly because it diverts resources from growth and reproduction. If algae can control their production of secondary metabolites, costs of production can be deferred until herbivores are detected, at which time the costs may be offset by the benefits of protection. This model predicts that plants subject to greater herbivory will have higher concentrations of secondary metabolites than plants subject to low levels of herbivory.

An alternative framework to differential herbivore regimes suggests that algal chemical defenses may be influenced by resource availability. The resource-availability

model (Hay 1996) postulates that secondary metabolites are a function of carbon availability relative to other nutrients. If algal growth and reproduction are limited by nutrients other than carbon, excess carbon may be stored as secondary metabolites. This model predicts that algae growing in environments where carbon is limiting (e.g., areas of low irradiance) should have lower concentrations of secondary metabolites than plants that are limited by other nutrients. Another resource-based model suggests that in resource-poor or -limited habitats (e.g., deeper water, low irradiance), plants should exhibit high resistance to herbivores because losing biomass is relatively more costly to a plant's fitness than in resource-rich environments (Coley et al. 1983).

Finally, physical stress can influence chemically-mediated herbivore defense in algae. Cronin and Hay (1996) found that both desiccation and exposure to ultra-violet (UV) radiation reduced concentrations of secondary metabolites in *Dictyota ciliolata*. Little is known about how the impact of UV radiation may vary across individuals from different depths. However, concentrations of photoprotective pigments, commonly referred to as mycosporine-like amino acids (MAAs), are higher in coral colonies growing in shallow water than in colonies from deep water (Shick et al. 1995), and a preliminary survey of MAAs in marine organisms found that they were present in numerous species of algae (Banaszak et al. 1998).

This study investigated intraspecific variation in herbivore defense in two algae species that are common on the forereef of Discovery Bay, Jamaica. *Dictyota divaricata* (phaeophyta) and *Lobophora variegata* (phaeophyta) are macroalgae that are abundant at deeper depths ( $\geq 15$  m) but also occur in shallower water ( $< 5$  m) on the west forereef (Morrison 1988). Both species produce polyphenolics, secondary compounds that are

analogous to tannins in terrestrial plants (Hay and Fenical 1988). However, as far as we know there is no documentation of intraspecific variability in the production of these compounds in these two species.

We hypothesized that depth would influence herbivore defense in *D. divaricata* and *L. variegata*. The spatial-variation-in-herbivory and the resource-availability models both predict that individuals from shallower depths would be better defended (1) because herbivory tends to decline with depth on this reef (Morrison 1988), and (2) because the penetration of photosynthetically active radiation (PAR, 400 – 700 nm) is greatly attenuated by sea water (Dunne and Brown 1996). Alternatively, the relative costs of herbivory to fitness may be greater in light-limited deeper environments, and therefore individuals in deep water may be better defended against herbivores. We also hypothesized that exposure to increased levels of UV radiation would reduce the defenses of the two species, and that individuals from deeper water with potentially lower concentrations of MAAs may show a greater vulnerability to herbivory than individuals from shallow water.

## METHODS

All data were collected between 6 and 10 March 2000 at the Long Term Study (LTS) site in Discovery Bay, Jamaica, West Indies.

### Depth-Dependent Herbivory Experiments

Twenty individuals of both *D. divaricata* and *L. variegata* were collected at three depths (5 m, 10 m, and 15 m). *L. variegata* occurs in three different morphotypes, but we sampled only the shelf form of this species (Littler et al. 1995). Algae samples were taken to the laboratory where they were separated into approximately equal portions, patted dry on paper towels and weighed (fresh mass). Each replicate was composed of three individuals

of a single species, each from a different depth, attached to a bolt with a wooden clothespin. Replicates were placed along a transect at a depth of 5 m, where *D. antillarum* abundances were greatest, at approximately 15:00 and retrieved after 48 h and re-weighed in the laboratory. We tested the effect of the depth at which algae were collected on percent biomass lost to herbivory using an ANOVA blocked by replicate to control for variability between replicates. Orthogonal contrast was used to further investigate the effect of depth on herbivory of *D. divaricata*.

#### Ultra-Violet Radiation Experiments

We collected five individuals of *D. divaricata* at three depths (5 m, 10 m, and 15 m). Samples were placed in herbivore exclosures, made from 1000 cm<sup>3</sup> plastic tubs with chicken wire covers, and placed at a depth of 1 m in the water near the laboratory in order to increase the exposure to UV radiation. After 48 h the algae were taken to the laboratory where they were separated into approximately equal portions, patted dry on paper towels and weighed (fresh mass). Each portion of algae was then attached to a bolt with a wooden clothespin and paired in replicates with freshly retrieved algal samples from the same depths (5 m, 10 m, 15 m) on the reef. Each replicate was comprised of two plants: one that had been exposed to UV radiation (i.e., in < 1 m of water) and one recently removed from natural conditions on the reef. Replicates were placed at a depth of 5 m, collected after 48 h, and re-weighed in the laboratory. Two controls were tested: one collected at the same time as the experimental algae exposed to UV, placed in running seawater laboratory; and one freshly collected on the morning of placement. Control samples from the laboratory were not used in the analysis as they experienced the same amount of herbivory as fresh controls but with more variability between replicates. *L. variegata* was

not tested for effects of UV exposure because samples were lost from the herbivore exclosure tubs. The effects of depth, UV exposure and depth x UV exposure interaction on percent biomass lost to herbivory was analyzed using a two-way ANOVA blocked by replicate to account for variability between replicates.

#### Algae and Urchin Composition and Distribution

We conducted a survey of algal types at 5 m intervals from the forereef crest at depths from 1 m to 15 m. Algae were classified by the following functional groups: crustose corallines, filamentous turf-forming algae (finely branched or unbranched species generally < 2 cm tall; Morrison 1988), and erect macroalgae. Percent coverage of these types was determined at each meter along four 30-m transects at each depth by a point intercept method, and pooled estimates of percent algal cover were calculated from the sum of these transects. Additionally, we noted the occurrence of our two focal species, *D. divaricata* and *L. variegata*, within the macroalgae functional group.

We also conducted a survey of urchin abundance at the four depths. At each depth, we sampled twenty-five 1-m<sup>2</sup> quadrats at 2 m intervals along a transect, and all urchins within the quadrats were recorded. Urchin abundances were non-normal despite transformation, so the abundances by depth were analyzed with a non-parametric Kruskal-Wallis rank sums analysis.

### RESULTS

#### Depth-Dependent Herbivory Experiments

Herbivores showed variable preference for *D. divaricata* and *L. variegata* depending on the depths from which algal samples were taken (Fig. 1). *D. divaricata* from deeper depths lost significantly more biomass during the study period (Table 1); samples taken from

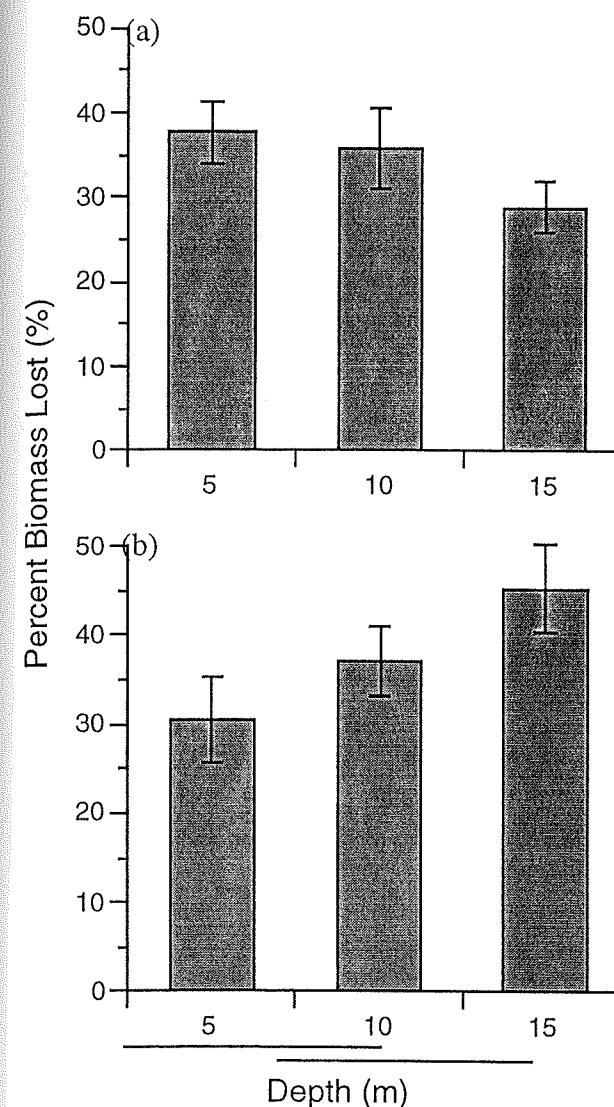


Figure 1. Percent of (a) *L. variegata* and (b) *D. divaricata* biomass lost to herbivory over 48 hours at three depths on the forereef of Discovery Bay, Jamaica. Values are means  $\pm$  SE. Horizontal bars below graph (b) indicate depths that are not significantly different for *D. divaricata* by orthogonal contrast analysis.

15 m lost significantly more biomass to herbivory than those taken from 5 m (orthogonal contrast,  $p = 0.01$ ). The opposite pattern was apparent for *L. variegata* (Table 2), though the trend was not statistically significant.

#### Ultra-Violet Radiation Experiments

Exposure to UV radiation increased susceptibility to herbivory in *D. divaricata* (Table 3). UV exposed treatments tended to

Table 1. Effects of depth (samples taken from 5 m, 10 m, and 15 m) and the blocked effect of replicate on percent removal of *L. variegata* by urchin herbivory in Discovery Bay, Jamaica.

Source	DF	MSE	F-ratio	P
Depth	2	410.5	1.62	0.21
Replicate	18	364.3	1.44	0.17
Error	36	253.5	-	-

be more susceptible to herbivory as depth increased (Fig. 2). It is not surprising that the controls in the UV experiment do not follow the same trend found in the depth-dependent herbivory experiments, because controls from the 3 depths were not placed within the same replicate in the UV experiment. Therefore, urchins were not presented with the choice between different depths and differences may not be as apparent as those in trials of direct preference.

#### Algae and Urchin Composition and Distribution

Total algal cover fluctuated between 50 and 85% among depths but appeared to be more abundant at deeper depths (Fig. 3). Macroalgae, the dominant functional group across all depths, became dramatically more abundant as depth increased; conversely, crustose algae decreased in cover as depth increased. Turf algae accounted for the least algal cover in our transects, reaching a maximum of 10% cover at 10 m. *D. divaricata* and *L. variegata* were both abundant algal species, accounting for up to 50% of the algal cover at 15 m. *D. divaricata* was generally more abundant than *L. variegata* at all depths, although both species increased in percent cover with increasing depth (Fig. 4).

Our urchin survey yielded four species of urchins (Table 4). Total urchin abundance decreased significantly with increasing depth (Kruskal-Wallis Rank Sums,  $X^2 = 50.46$ ,  $df = 3$ ,  $p < 0.001$ ), with maximum abundance occurring at a depth of 5 m (Fig. 5). *Diadema antillarum* dominated the urchin population at shallow depths, and accounted for nearly



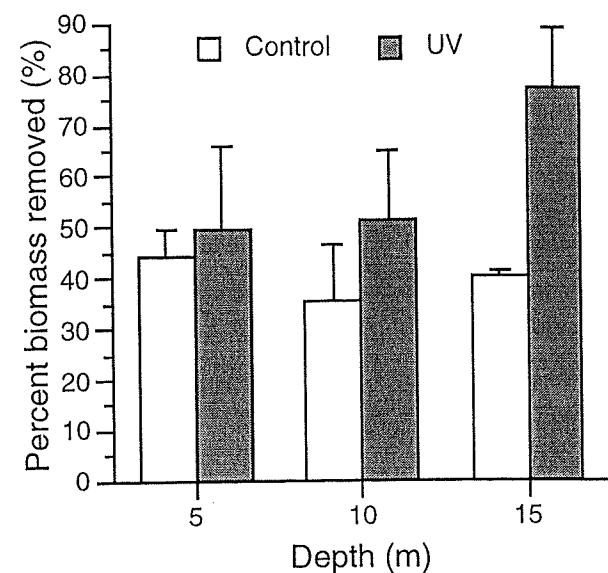


Figure 2. Effect of UV level and depth of sample collection on percent biomass lost to herbivory in *D. divaricata*. All replicates were placed at a depth of 5 m for 48 hrs at the LTS site, Discovery Bay, Jamaica. Values are means  $\pm$  SE.

all urchins found in the survey (Table 1, Fig. 5).

#### DISCUSSION

The effect of depth on algal susceptibility to herbivory differed distinctly between *L. variegata* and *D. divaricata*. While *L. variegata* samples from 5 m were consumed more than those from 15 m, the opposite pattern existed for *D. divaricata*. These results suggest that patterns of herbivore defense may be species-specific and that herbivore abundance, light attenuation, and relative metabolic costs may all be important factors in regulating levels of chemical defense in macroalgae, though the importance of their respective roles may vary across species.

Herbivory trials with *L. variegata* from different depths indicated a trend that individuals from shallow depths may be more susceptible to herbivores than those from deeper depths. Although not statistically significant, this trend suggests that relative metabolic costs may be important in determining

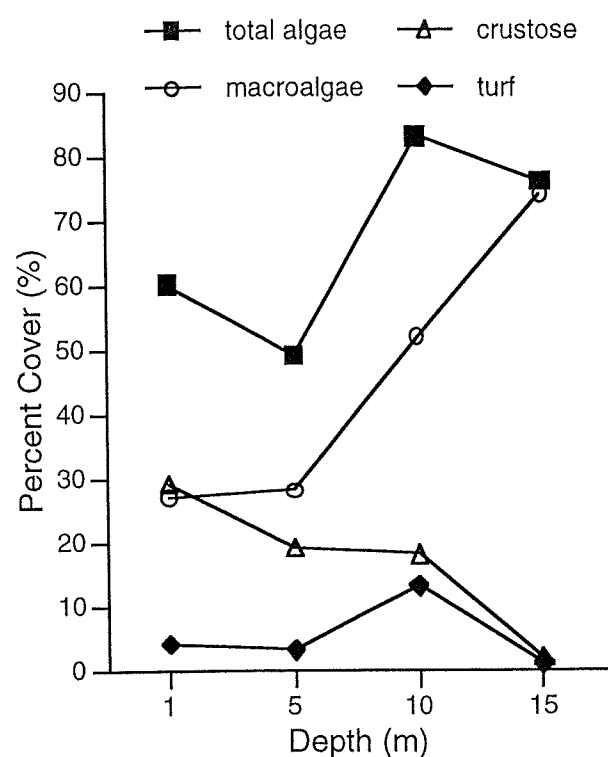


Figure 3. Percent algal cover at LTS, Discovery Bay, Jamaica. Percent cover was determined by point intercept method every 1 m along 120 m transects at each of 4 depths.

the amount of energy invested in defense in *L. variegata*. Individuals growing in resource-limited environments (e.g., greater depths with low irradiance) may be better defended than individuals from resource-rich (e.g., shallow, high irradiance) environments because the benefits of protection for these nutrient-limited populations are relatively greater. Biomass lost to herbivory in deeper waters would be a larger proportion of net growth compared to the same loss in shallow waters, and would take longer to replace (Hay 1981), therefore increasing the relative benefit of herbivore defense.

That individuals from deeper water may be less susceptible to herbivory is surprising, however, given that individuals growing at a depth of 15 m likely face much lower levels of herbivory than individuals growing at 5 m. Urchins, the primary herbivores of *L. variegata* on this reef (Morrison 1988), decrease

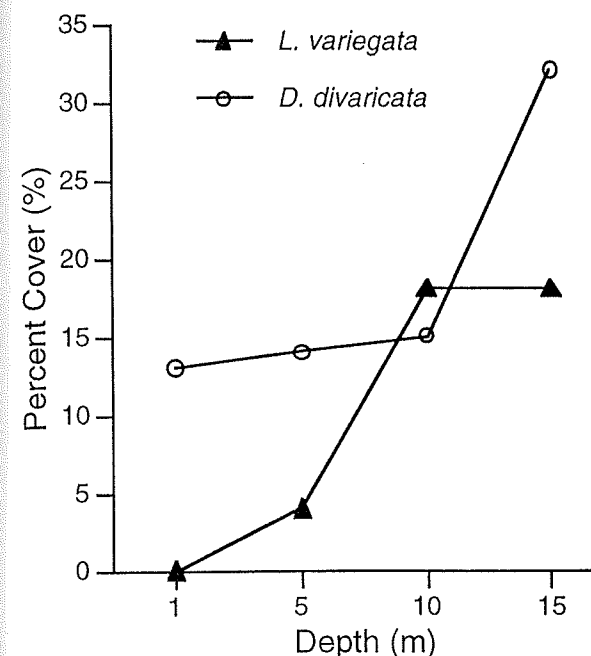


Figure 4. Percent cover of macroalgal species *L. variegata* and *D. divaricata* at LTS, Discovery Bay, Jamaica, determined in the same point intercept surveys conducted for Fig. 1.

in abundance with depth (Fig. 3). Therefore, there appear to be relatively few benefits of producing secondary metabolites at deeper depths. It is equally possible that *L. variegata* does not exhibit variation in secondary metabolite concentrations. The variety of forms of this species suggests that morphological variation may play an important role in defense against herbivory (Littler et al. 1989). If the calcified morphotype of this species is more abundant in areas of high herbivory, it would support the hypothesis that morphological defense is an important strategy employed by this species to reduce herbivory.

Contrary to the pattern of herbivory on *L. variegata*, *D. divaricata* from 15 m suffered greater losses to herbivory than individuals from 5 m. These results support both the spatial-variation-in-herbivory and the resource-availability hypotheses. The lower urchin abundance at 15 m than 5 m probably corresponds to lower herbivore pressure on individuals at deeper depths, thus reducing the necessity of secondary metabolite defenses.

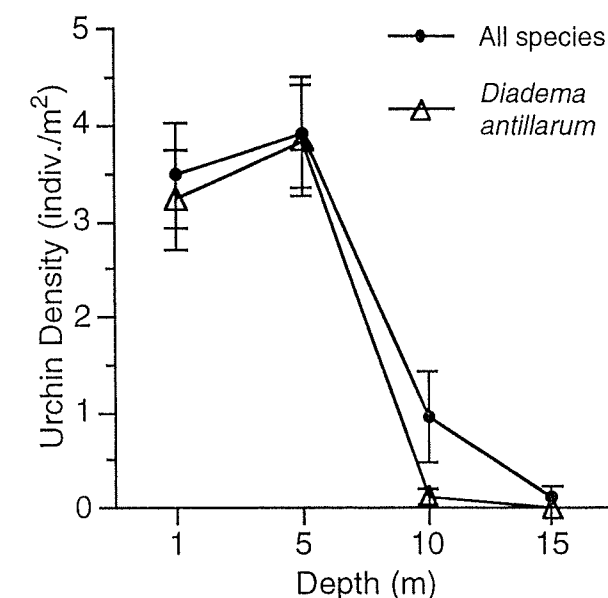


Figure 5. Urchin densities at 4 depths at LTS, Discovery Bay, Jamaica. Values are means  $\pm$  SE.

Alternatively, the resource-availability model suggests that the lower resource availability at increased depths, due to the dramatic attenuation of PAR (Dunne and Brown 1996), may limit excess carbon availability and therefore the synthesis of secondary metabolites. A possible test to determine which of these two mechanisms may be influencing herbivore defenses in *D. divaricata* would be to compare the susceptibility of individuals that come from similar resource conditions (e.g. same depth) but different herbivore regimes.

*D. divaricata* also differed in its susceptibility to herbivory after exposure to UV radiation. Stress-induced increases in algal palatability may result from decreases in either morphological or chemical defense. Individuals that were exposed to heightened levels of UV radiation showed signs of wilting but did not differ greatly in toughness or in general texture (pers. obs.). Therefore, it is likely that differences in herbivory between UV-exposed algae and controls may be due to compromised chemical defenses rather than changes in morphology. Cronin and Hay (1996) found a similar trend in *Dictyota ciliolata*. This species was relatively unpalatable in its un-

stressed state but became more palatable and exhibited a 43% reduction in chemical defenses when physically stressed by prolonged UV exposure. In our study this pattern was more pronounced in algae from 15 m than in algae from 5 m, suggesting that individual *D. divaricata* may differ in their protection from UV radiation. The most wide-spread UV protective compounds that have been identified in marine organisms are mycosporine-like amino acids (MAAs) which absorb a large proportion of UV radiation (Banaszak et al. 1998). The occurrence of MAAs follows a pronounced bathymetric distribution in some coral species, with significantly higher concentrations in individuals growing at 10 m than those growing at 20 m (Shick et al. 1995). Our results suggest that MAA concentrations, if present in *D. divaricata*, may follow similar patterns.

Our results indicate that herbivore defenses vary with depth (5 to 15 m) in *D. divaricata* and possibly in *L. variegata*. Patterns of intraspecific variation in herbivore defense appear to be species-specific and do not follow a general trend along this depth gradient. We also found that exposure to UV radiation significantly reduces herbivore defenses in *D. divaricata* individuals from deeper depths. Herbivore defense clearly depends on a variety of complex factors, and understanding their relative importance is essential to explain species distributions within coral reef ecosystems.

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